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ABSTRACT

This study reexamined the concept that delayed muscle soreness (DMS) is a form of inflammatory pain. This was accomplished by having 32 male volunteers perform exercise known to induce DMS and then assess the total and differential white blood cell changes. In addition, an anti-inflammatory drug, idomethacin, was administered to determine whether this would reduce the perception of muscle soreness. The increase in neutrophils and decrease in DMS with drug administration suggested that inflammation is involved in DMS. (JD)

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NEUTROPHILIA AND AN ANTI-INFLAMMATORY DRUG AS MARKERS OF INFLAMMATION IN DELAYED MUSCLE SORENESS

Key words: inflammation, neutrophils, muscle soreness, indomethacin

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Thirty-two untrained males were assigned to an experimental (E) or a control group (C) to re-examine the concept that delayed muscle soreness (DMS) represents an acute inflammatory response. Subjects were assigned to receive either Indomethacin (Id; 100 mg per day) for 2 days prior to the treatment and a placebo (P) for 2 days after (Id-P); or the reverse combination (P-Id); or Id for 4 days (Id-Id); or placebo (P-P) for 4 days. On the treatment day, E subjects performed 30 min of bench stepping to induce DMS; C subjects rested for 30 min. Immediately before and after stepping/resting, all subjects used their right and left legs to perform 19 maximal contractions on the Cybex II. Blood samples were collected -5 min before, and then after bench stepping at 0, 2, 24, 48, and 72 h, to evaluate white blood cell (WBC) response. DMS was also monitored at 0, 24, 48, and 72 h. All E subjects experienced a significant amount of DMS ($p<.01$); however, Id-Id and P-Id experienced significantly less ($p<.05$) soreness than P-P. Total WBC count (cells/mm) was significantly greater at 0 h (8,340 $\pm$ 380) than at -5 min (6,699 $\pm$ 365) for both E and C; this increase was most likely a response to the isokinetic exercise. At 2 h there was a significant increase in total WBC count for E (9,603 $\pm$ 389) and no change for C (8,336 $\pm$ 273). Neutrophils increased significantly at 2 h for E only (6,428 $\pm$ 375 vs 4,988 $\pm$ 261 for C). The increase in neutrophils and decrease in DMS with drug administration suggests that inflammation is involved in DMS.

INTRODUCTION

It is well established that eccentric contractions of sufficient intensity and/or duration (26) result in delayed muscle soreness (DMS) (13, 20). DMS is initially perceived between 8 and 24 hours after exercise, peaks between 24 and 72 h, and then rapidly declines (12). It is also well documented that such eccentric contractions result in cellular disruption of involved musculature (2, 3, 13). Although evidence is inconclusive, it is believed that this disruption is related to DMS (2). However, the biochemical events which link the cellular disruption to the onset of DMS a day or so later are not well understood.

At one point it was proposed that DMS was related to the inflammatory process (27, 28); this theory has generally been disregarded (2, 3, 13, 24). One reason for rejection of this hypothesis was a lack of evidence of increased activity of leukocytic neutrophils (24). A significant increase in neutrophils in the peripheral circulation is an important systemic marker of chronic and acute inflammation (6, 21, 22). During acute inflammation this neutrophilic response occurs shortly after the onset of tissue injury, and is only detectible in the circulation for a brief period (6). It is possible that the sampling times used by previous researchers were not sufficiently sensitive to detect these acute fluxes (3, 14).

If DMS were a form of inflammatory pain it would be

reasonable to anticipate that an anti-inflammatory drug would decrease soreness sensations. A limited amount of research has examined the affects of anti-inflammatory drugs on DMS and the results of these studies are contradictory (4, 18).

The objective of this study was to reexamine the concept that DMS is a form of inflammatory pain. This was accomplished by having subjects perform exercise known to induce DMS and then assess the total and differential white blood cell (WBC) count at times deemed more appropriate for detecting these changes. In addition, an anti-inflammatory drug, indomethacin, was administered to determine whether this would reduce the perception of muscle soreness.

METHODS

Subjects. Thirty-two healthy male volunteers with an age range of 18 to 35 years agreed to participate in this study. None of the subjects participated in a regular program of physical activity all were screened to insure no previous allergic reaction to aspirin-like drugs. The VO2 max was measured for each subject prior to the bench stepping/resting condition using a standard Bruce protocol on a motor driven treadmill (1). To determine body fat, three skinfolds were measured using the Jackson and Pollock generalized equation (17). Informed consent was obtained from all subjects; this was in accordance with the policy statement of the American College of Sports Medicine and the Institutional Human Subjects Committee.

Insert table 1 about here

Experimental Design. The initial 16 subjects screened were assigned to an experimental bench stepping condition (E). These subjects were required to perform one continuous 30 min bout of bench stepping at a rate of 15 step-ups/min; each contraction lasted 1 second with the cadence determined by an electronic metronome. Throughout the step test the quadriceps of one limb contracted concentrically by leading the step-up, whereas the contralateral quadriceps muscle group contracted eccentrically by controlling the step-down. The bench height was adjusted for each subject so that the effort was controlled at approximately 57% of the subject's aerobic capacity. However, if a subject's heart rate exceeded 160 beats/min at 9 min, the bench was lowered by 7.5 cm to ensure that the subject would complete the 30 min of stepping. Sixteen more subjects were subsequently screened. This group was designated the control group (C). Instead of bench stepping, C subjects remained seated in the laboratory for 30 min.

Drug Administration. For four days all subjects in E and C received an oral dose of either a placebo (P) or indomethacin (Indocin, provided by Merck, Sharp and Dohm; maximum dose = 25 mg four times each day), or a combination thereof. Drug or

placebo admininstration began 36 h prior to stepping/resting and continued for 60 h after. A double-blind design was used to assign E (N=16) and C (N=16) subjects to one of the following drug groups: Id-Id group received Indocin for 4 days (total dose = 400 mg); P-P received placebo for 4 days; Id-P received Indocin for the initial 36 h (total dose = 150 mg) and placebo for the final 60 h; P-Id received placebo for the initial 36 h and Indocin for the final 60 h (total dose = 250 mg).

Cybex II Testing. Immediately before and after the 30 min of bench stepping/resting, as well as at 24, 48, 72 h after, all subjects in E and C were tested on the Cybex II machine. Subjects were required to use each lower extremity to perform eight maximal and five submaximal contractions at 60 degrees sec as well as eleven maximal and ten submaximal contractions at 250 degrees sec. This Cybex testing actually represented a separate study and results are reported elsewhere (29). The reason for including this procedure in the methodology, is that this short intense bout of exercise appeared to have an impact on the WBC count.

Total and Differential White Blood Cell Count. Six blood samples were collected for the 16 E subjects and 16 C subjects (-5 min before and then 0, 2, 24, 48 and 72 h after stepping/resting). On each occasion, approximately 3 ml of blood were collected from an antecubital vein into evacuated blood collection tubes containing EDTA. Slides of whole blood were prepared and then analyzed using a Technicon 6000

autoanalyzer; approximately 10,000 consecutive cells were counted and a total and differential WBC count was computed for each sample.

Perception of Muscle Soreness. At 0, 24, 48 and 72 h after bench stepping, all subjects reported their perception of DMS in the vastus medialis, vastus lateralis, gluteus, adductors, calf and "other" muscles (anywhere else), for both extremities. Subjects were asked to rate the amount of soreness or pain during palpation with reference to Henry's Pain Scale which has a range from 0.0 to 6.75 with 0.25 gradations (16). A "composite-pain score" was then computed for each subject on each occasion; this was the simple arithmetic sum for that test interval. As C subjects did not experience any DMS, soreness scores were not computed for them.

Statistical Analysis. To analyze changes in total and absolute WBC counts a 3-factor design, i.e., treatment, drug, and time (2 x 4 x 6) with repeated measures on the third factor, was used. To evaluate DMS experienced by E subjects a 2-factor design (4 x 4) with drug and time as the factors and with repeated measures on the last factor, was used. The Tukey-Kramer test was used to perform all post hoc tests. All values were tested for significance at the alpha =.05 level.

RESULTS

Because of problems with certain blood samples, such as decomposed cells, total and differential WBC counts were analyzed for 13 of 16 E and 14 of 16 C subjects (Fig. 1). When

total WBC count at 0 h was compared to baseline there was a 22% and 18% increase ($p < .01$) for both E and C, respectively. At 2 h E showed a further 14% increase ($p < .01$); this was significantly greater ($p < .01$) than that of the C at 2 h. Lymphocytes, at 0 h , increased 31% for E and 29% for C ($p < .01$) over baseline levels (Fig. 1). Neutrophils for the E increased 39% ($p < .01$) at 2 h, compared to baseline; no significant increases in neutrophils were observed at any stage for the C. The only drug effect observed was on monocytes ($p < .05$) where a three way interaction between treatment, drug and time was seen.

Insert figure 1

All bench stepping subjects experienced a significant amount of soreness ($p < .01$). DMS was first noted at 24 h when the mean cumulative score for all four drugs groups was 5.8; by 48 h the intensity of soreness had increased by 34% to 8.8 and this represented the peak intensity of soreness. Between 48 h and 72 h there was a 55% decrease in perception of soreness to a mean level of 3.9.

Insert figure 2

For most subjects soreness was not confined to one muscle group. Thirty-two percent of the E subjects experienced peak DMS predominantly in the medialis muscle of the quadriceps group of the leg which supported the weight as the body was being lowered from the bench. Fifty percent of the subjects experienced the soreness predominantly in the calf muscles of the opposite leg which made the initial contact with the ground during the descent. The remaining subjects experienced peak muscle soreness in some "other" muscle group.

When the mean of each drug group was collapsed across time, there was a significant difference ($p < .05$) between the amount of soreness experienced by subjects in the drug groups (Fig. 2). Post hoc tests indicated that the subjects who had been administered a placebo for 4 days (P - P) experienced significantly more DMS than the group which had been administered either indomethacin for 4 days (Id - Id, $p < .01$) or indomethacin beginning 2 hours after the bench stepping (P - Id, $p < .05$).

DISCUSSION

Physical exercise has a rapid effect on circulating white blood cells (31). Endurance exercise generally leads to a preferential increase in neutrophils (30) whereas short-term exercise results in lymphocytosis (5). The similar increases for the C and E group seen in total WBC count and lymphocytes at 0 h, can most likely be attributed to the short, intense

bout of exercise performed on the Cybex II machine. Although the total WBC count for C remained elevated at 2 h the proportions of the various leukocyte fractions at this time were almost identical to the baseline values i.e., all were slightly elevated but none were significantly different from the baseline values.

A central objective of this study was to determine whether or not neutrophilia would occur after eccentrically biased exercise. Schwane et al (21) found a non-significant 18% increase in neutrophils after a bout of downhll running; similarly, our subjects demonstrated a non-significant 18% increase in neutrophils immediately after bench stepping. However, when blood samples were collected two hours after the termination of exercise, we did find a significant increase in neutrophil count.

Although, neutrophilia is a common feature of the inflammatory process, this condition may also occur in the absence of inflammation , i.e. in response to a variety of agents including glucocorticoids (20). Plasma cortisol has been shown to increase when individuals work at an intensity equal to or greater than 60% of their VO2 max (15). Furthermore, cortisol which has a plasma half-life of 60-90 min (9) has been shown to peak after the termination of exercise (15). In this study bench stepping was performed at an average intensity of 60% $\dot{V}O_2$ max capacity with a range of 52-62%; it is therefore conceivable that cortisol increased, and the observed neutrophilia at 2 h was a result of this increase.

To help determine whether neutrophilia was a response to cortisol or to inflammatory demands, an analysis of the overall leukocytic pattern may be helpful (31). It is well documented that for several hours following the administration of glucocorticoids, there is a dramatic increase in neutrophil count and a decrease in circulating lymphocytes, monocytes, and eosinophils (9, 31). On the other hand, the leukocytic response for the equivalent time period following the onset of acute inflammation shows a more moderate increase in neutrophils, an initial decrease followed by an increase in monocytes, a variable response in eosinophils and a decrease in lymphocytes (31). It appears that the overall leukocytic picture displayed by the E subjects at 2 h after exrcise, more closely resembles the response to acute inflammation as opposed to cortisol (Fig. 1).

By 24 h, most of the WBC fractions in the E and C group had returned to baseline levels and remained there through 48 and 72 h; this was generally consistent with the results of other researchers who evaluated leukocytes after exercise known to induce DMS (24). Some researchers have indicated a significant decrease in monocytes at 24 h (3) or 48 h (24) after exercise. Such a response was not observed in this study; but it should be noted that this could have been masked by the drug effect on the monocytes, i.e., indomethacin has been shown to increase monocyte count (7).

The time-frame observed in this study for the onset of DMS at 24 h, the peak intensity at 48 h and the dramatic decline at

72 h, agrees well with the literature (11, 25). Some researchers have found muscle soreness detectible at 2 h (10) and 8 h (8) and the peak intensity occurring as early as 24 h (14).

Indomethacin resulted in a significant reduction in DMS; this is consistant with results reported by Bansil et al (3) who found that a therapeutic dose of aspirin given for 2 days before and 2 days after a bout of eccentrically biased exercise, signifcantly reduced DMS. Furthermore, we found that indomethacin was most effective when administered both before as well as after the bench stepping, but was also effective when administered only after the exercise. Salminen and Kihlstrom (23) reported that indomethacin was effective in suppressing inflammation in the muscles of mice when administered after a bout of strenuous exercise.

In summary, neutrophilia was evidenced at 2 h after a 30 min bout of bench stepping; the composite leukocytic pattern at this time suggested that this is a response to inflammatory demands. This response was not seen in the C group at 2 h. Furthermore the E subjects experienced DMS, which responded to the administration of an anti-inflammatory drug. These results suggest that DMS represents inflammatory pain.

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SUBJECT CHARACTERISTICS

	BENCH STEPPING SUBJECTS (E) (N = 16)	RESTING SUBJECTS (C) (N = 16)
Age (yr)	26.2 ± 4.1	25.4 ± 1.2
Weight (kg)	76.1 ± 2.7	72.8 ± 2.7
Body Fat (%)	11.4 ± 1.4	14.1 ± 1.8
VO2 Max (ml.kg-1.min-1)	41.5 ± 1.2	39.9 ± 1.9
Relative Workload During Stepping (% of VO2 Max)	56.7 ± 1.7	

Values are means ± standard error

FIGURE LEGEND

FIG. 1. Total white blood cell count and differential for experimental and control subjects. Values represent means and standard error of means (**p < .01 compared to pre-exercise).

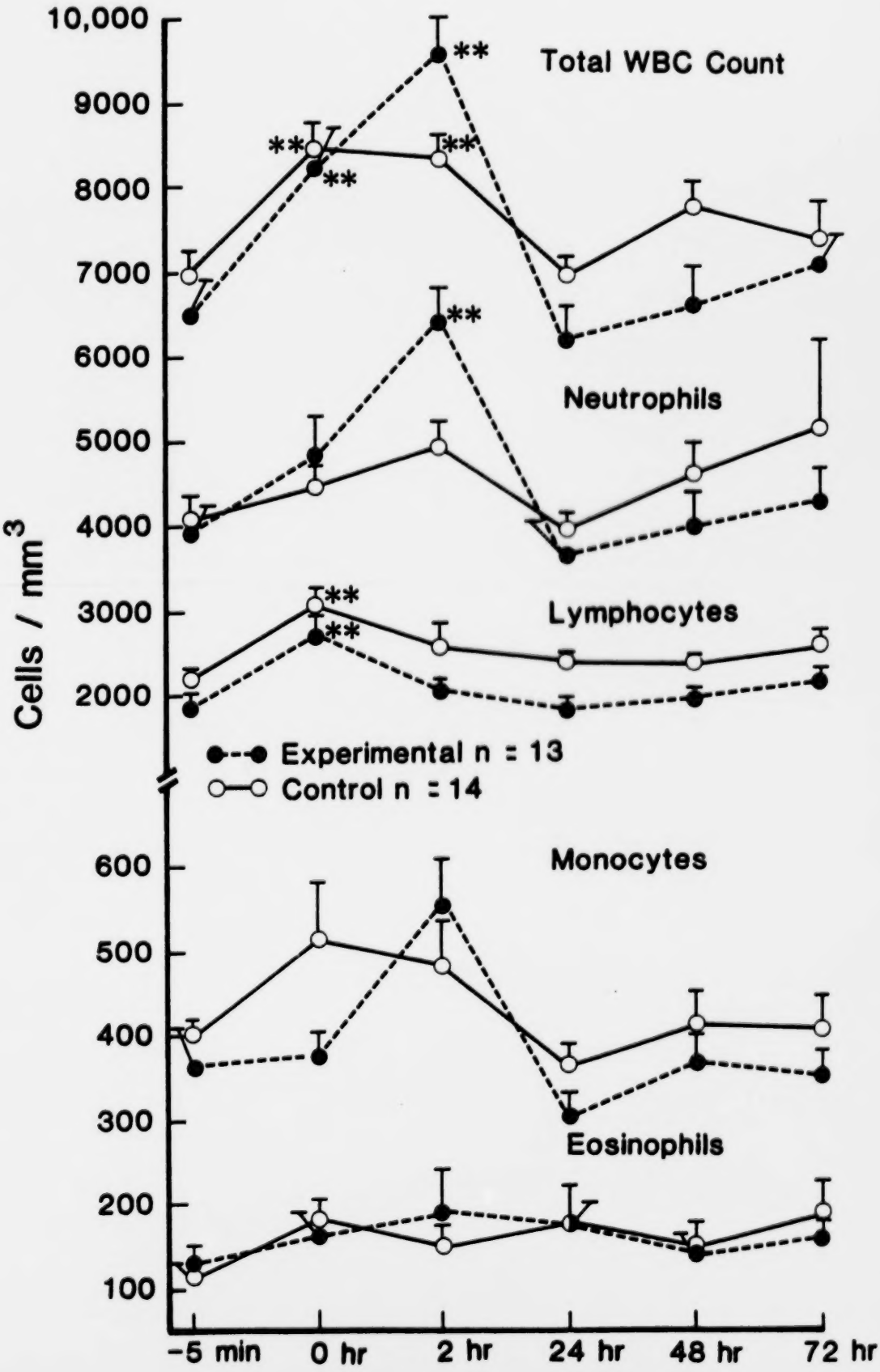
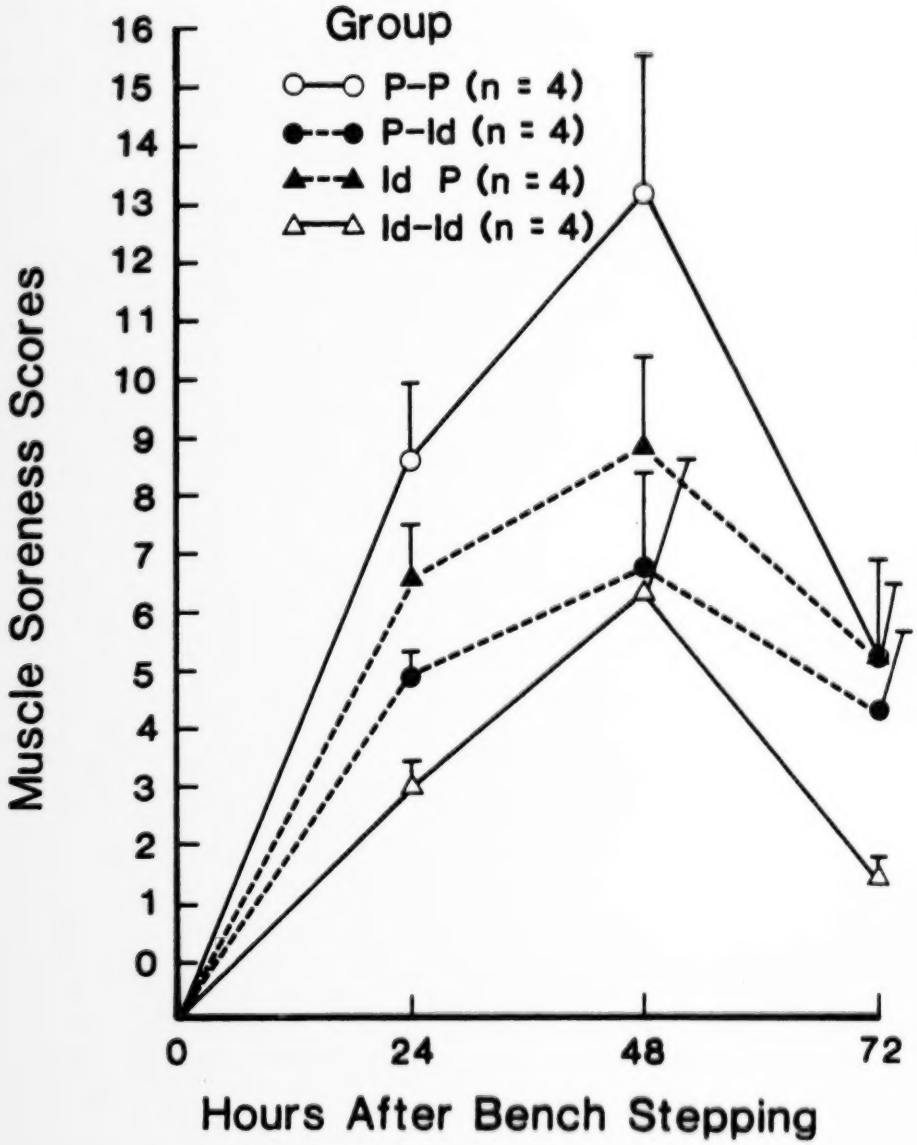

10,000
9000
8000
7000
6000
5000
4000
3000
2000
600
500
400
300
200
100
Cells / mm3
Total WBC Count
Neutrophils
Lymphocytes
Monocytes
Eosinophils
**
Experimental n = 13
Control n = 14
-5 min
0 hr
2 hr
24 hr
48 hr
72 hr
Time After Blood Sample

FIGURE LEGEND

FIG. 2. Composite muscle soreness scores for bench stepping subjects. Values represent means and standard error of means. P-P, placebo-placebo; P-Id, placebo-indomethacin; Id-P, indomethacin-placebo; Id-Id, indomethacin-imdomethacin.


Group
P-P (n = 4)
P-Id (n = 4)
Id P (n = 4)
Id-Id (n = 4)
Muscle Soreness Scores
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
24
48
72
Hours After Bench Stepping